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Testosterone Therapy in Adult-Onset Testosterone Deficiency: Hematocrit and Hemoglobin Changes

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Abstract

Objective: Hematocrit (HCT)/hemoglobin (Hb) ratio in (%/g/dL) is around 3, with high fidelity between measured and derived Hb (applying the conversion using HCT) in various pathologies. We examined changes in HCT and Hb values and HCT/Hb, compared with baseline, in men with adult-onset testosterone deficiency (TD) given testosterone therapy (TTh).

Materials and Methods: Data were analyzed from an observational, prospective registry study at various time points in 353 men with adult-onset TD receiving testosterone undecanoate (median follow-up: 105 months). After establishing baseline HCT/Hb, we compared (cf. baseline) changes in HCT, Hb, and HCT/Hb at 12, 48, 72, and 96 months. Regression analyses determined predictors of HCT and Hb change.

Results: TTh was associated with ($p < 0.0001$) increases in median HCT and Hb; 44% to 49% and 14.5 to 14.9 g/dL at final assessment, respectively. Regression analyses showed that HCT change was associated with baseline HCT and testosterone levels, while Hb change was associated with baseline Hb, HCT, and testosterone levels. In the total cohort and subgroups, HCT/Hb increased significantly at all time points ($p < 0.0001$, cf. baseline) with over 90% of men demonstrating increases. Linear regression showed that the ratio of HCT change/Hb change (i.e., difference between HCT at the various time points and baseline value/difference between Hb at the various time points and baseline value), following TTh at each time point was higher than the baseline HCT/Hb ratio.

Conclusion: HCT increase was greater than we anticipated from the established HCT/Hb of 3. We speculate that increased erythrocyte life span with associated higher Hb loss via vesiculation could account for our observation. This could have a bearing when using HbA1c as an indicator in men with adult-onset TD on TTh.

Keywords: testosterone therapy; hematocrit; hemoglobin; adult-onset testosterone deficiency; erythrocyte life span

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Introduction

Adult-onset testosterone deficiency (TD) is defined by low serum testosterone levels and associated symptoms and signs.[1] The condition is common, with a prevalence of 6–12% in the general male population and even higher at 40% in men with type 2 diabetes (T2DM).[2,3] A meta-analysis of pooled observational studies demonstrated increased all-cause and cardiovascular mortality in men with adult-onset TD.[4] Longitudinal studies by Muraleedharan et al.[5] and Hackett et al.,[6,7] having demonstrated increased mortality in men with T2DM and low serum testosterone, showed a reduction in all-cause mortality following testosterone therapy (TTh). A few studies[8–10] have suggested increased TTh-associated cardiovascular disease (CVD) and, despite the methodology being criticized,[11] organizations such as the U.S. Food and Drug Administration have expressed concerns.[12] Reassuringly, a meta-analysis of interventional studies concluded that appropriate TTh was not associated with increased risk of CVD, and in some subpopulations, a beneficial effect was possible.[13] Thus, guidelines by the British Society for Sexual Medicine[3] and International Society for Sexual Medicine[14] suggest that men with a serum total testosterone (TT) <8 nmol/L or free testosterone <0.180 nmol/L usually require TTh, while men with serum TT between 8 and 12 nmol/L may, depending on symptoms, be considered for a TTh trial.

Despite the accumulating safety data,[11,13] it is important that vigilance is maintained, especially for factors associated with CVD. This must be extended to subgroups as heterogeneity may be evident in adult-onset TD.[15] An elevated hematocrit (HCT) appears the most frequent adverse effect of TTh.[16,17] The relationship between HCT, atherogenesis, and mortality is not well understood, with many conflicting studies.[18–23] Interestingly, one of these showed a U-shaped association between HCT and mortality, suggesting a complex nonlinear relationship.[22] Currently, monitoring of HCT is recommended during TTh, with guidelines setting differing HCT thresholds (50–54%) above which change in management is recommended.[1,3,24–26]

The mechanism of testosterone-induced erythrocytosis has not been fully elucidated. While some early studies suggested indirect bone marrow action via erythropoietin activity,[27,28] these findings have not always been evident.[29] A more recent study in 2016 by Dhindsa et al. showed that TTh in men with hypogonadotropic hypogonadism increased HCT and this was associated with a rise in erythropoietin, expression of ferroportin and transferrin receptor-2, as well as suppression of hepcidin.[30] Testosterone leading to direct stimulation of bone marrow erythroblast synthesis and iron incorporation into the erythroblasts via circulating soluble transferrin receptor (sTfr), which is involved in the intracellular transport of iron and chiefly found within erythroblasts, have also been considered as possible mechanisms.[31–33] However, Coviello et al. were unable to demonstrate sTfr correlating with testosterone levels.[29]

Another putative mechanism for the observed erythrocyte increase associated with TTh could arise from a decrease in degradation. Following TTh, changes in lipid membrane composition of the erythrocyte have been observed, thereby perhaps enhancing erythrocyte flexibility and thus survival.[34] Interestingly, in healthy subjects, about 20% of erythrocyte hemoglobin (Hb) appears to be shed via vesiculation, a phenomenon that increases during the second half of the erythrocyte life span.[35,36] Thus, in the event of increased erythrocyte life span with a consequent greater Hb loss, an increased HCT/Hb ratio may be expected. In most individuals, the HCT (percentage) appears about three times the Hb (g/dL), with high fidelity between measured and derived (from HCT) Hb values.[36] Furthermore, sensitivity, specificity, and positive predictive values (except in anemia) remained high, irrespective of age, gender, renal function, and hydration status.[37–39]

In this analysis, we aim to characterize Hb and HCT changes (absolute values and the ratio) associated with TTh in men with adult-onset TD, using data from an observational registry study[40] at various time points (0, 12, 48, 72, and 96 months). First, the aim was to report changes in HCT and Hb, and second, to examine the HCT/Hb ratio at each time point (in the total cohort and in subgroup-stratified baseline characteristics).

Materials and Methods

The data used were from an observational, prospective, cumulative registry study[40] of 353 men [median age (IQR): 60.0 (55.0, 64.0), median follow-up (IQR): 105 (78, 141) months] with adult-onset TD (serum TT ≤12.1 nmol/L) given testosterone undecanoate (TU) 1000 mg/12 weeks following an initial 6-week interval). Data were collected at a minimum of 6-month intervals. The database also contained 384 men [median age (IQR): 64.0 (60.0, 67.0), median follow-up (IQR): 114 (96, 126) months] who opted against TTh due to financial constraints and/or negative perceptions of

TTh. The main analyses studied changes in HCT, Hb, and HCT/Hb ratios after 12 (353 men), 48 (313 men), 72 (279 men), and 96 (207 men) months of TTh with the number of patients decreasing in view of the study design. The baseline characteristics of the 353 men commenced on TU are shown in Table 1 and footnotes. The German Medical Association's ethical guidelines for observational studies were adhered to with every participant consenting to be included and having his data analyzed. Following review, ethics committees in Germany and England stated that formal approval was not required. Institutional review board statement for University Hospitals Birmingham was received.

Serum TT (trough) levels were measured using an immunoassay (Abbott Architect). Hb levels were checked using photometry (CELL DYN Ruby/Abbott) and HCT was estimated using Microhematocrit (Mindray 3000 Plus).

Statistical methods

The baseline HCT and Hb values were not normally distributed with both skewness and kurtosis evident ($p < 0.0001$ when considered in combination), hence nonparametric tests were used to compare changes in HCT, Hb values, and HCT/Hb ratios between baseline and fixed time points during treatment. Sign-rank tests were carried out to compare changes in HCT and Hb values between baseline and at fixed time points during treatment. Factors associated with change in HCT and Hb during follow-up were studied using multiple regression. HCT/Hb ratios were calculated for each individual, and changes between the baseline values and those obtained after 12, 48, 72, and 96 months of TTh were compared using sign-rank tests. Finally, the associations between (1) baseline HCT and Hb, and (2) changes in HCT and changes in Hb were studied using linear regression, with scatterplots with trend lines visually reinforcing the findings.

Results

Table 1 shows that serum TT levels increased ($p < 0.0001$, sign-rank test) from baseline (median: 10.05 nmol/L) to 16.64, 16.99, 15.95, 16.99, and 18.72 nmol/L at 12, 48, 72, 96 months, and final assessment, respectively, in the men receiving TTh. In the men not receiving TTh, median serum TT was 9.71 and 8.32 nmol/L at baseline and final assessment, respectively. HCT or Hb did not increase during follow-up in the 384 men not opting for TTh; median (IQR) HCT = 46 (45–47) % and median (IQR) Hb = 14.7 (14.3–15.1) g/dL at baseline; median (IQR) HCT = 46 (45–47) % and median (IQR) Hb = 14.5 (14.2–15.0) g/dL at final assessment. In contrast, TTh was associated with an increase ($p < 0.0001$, sign-rank test) in both HCT and Hb values in the 353 treated men;

Table 1. Hematocrit and hemoglobin values at baseline and fixed time points (12, 48, 72, and 96 months of testosterone therapy) in the total cohort and subgroups stratified by smoking and type 2 diabetes

	Pre-TTh	12 months TTh	48 months TTh	72 months TTh	96 months TTh
			Median (IQR)		
Total cohort, *n*	353	353	313	279	207
TT (nmol/L)	10.05 (9.36–10.75)	16.64 (14.91–19.07)	16.99 (15.94–19.07)	15.95 (14.91–17.68)	16.99 (15.95–18.38)
Hb (g/dL)	14.5 (14.1–14.9)	14.7 (14.3–15.3)	14.8 (14.6–15.3)	14.9 (14.6–15.3)	15.1 (14.7–15.3)
HCT (%)	44 (43–46)	46 (45–48)	48 (47–49)	48 (47–49)	48 (47–49)
Cohort categorized by baseline characteristics					
Current smokers, *n*	135	135	119	103	88
Hb (g/dL)	14.6 (14.2–15.1)	14.9 (14.5–15.4)	15.0 (14.6–15.3)	15.1 (14.7–15.3)	15.2 (14.7–15.4)
HCT (%)	44 (43–45)	47 (45–49)	48 (47–49)	48 (47–49)	48 (47–49)
Nonsmokers, *n*	218	218	194	176	119
Hb (g/dL)	14.5 (14.1–14.8)	14.7 (14.3–15.2)	14.8 (14.6–15.3)	14.8 (14.6–15.3)	15.0 (14.7–15.3)
HCT (%)	44 (43–46)	46 (45–48)	48 (47–49)	48 (47–49)	48 (47–48)
Men with T2DM, *n*	148	148	121	100	74
Hb (g/dL)	14.6 (14.2–14.9)	14.8 (14.5–15.3)	14.9 (14.7–15.3)	15.0 (14.7–15.3)	15.2 (14.8–15.3)
HCT (%)	45 (44–46)	46 (45–48)	48 (47–49)	48 (47–49)	48 (47–49)
Men without T2DM, *n*	205	205	192	179	133
Hb (g/dL)	14.3 (14.1–14.8)	14.7 (14.3–15.3)	14.8 (14.5–15.2)	14.8 (14.5–15.3)	15.0 (14.6–15.3)
HCT (%)	44 (42–45)	46 (44–48)	48 (47–49)	48 (47–49)	48 (47–49)

Baseline characteristics of the 353 men not shown in the above table; median (IQR). Age: 60 (55, 64) years, follow-up: 105 (78, 141) months. Waist circumference: 108 (100, 114) cm. Serum TT: 10.05 (9.36, 10.75) nmol/L. HbA1c: 8.15 (5.8, 8.9)%, total cholesterol: 7.7 (7.2, 8.6) mmol/L, triglycerides: 3.2 (2.8, 3.5) mmol/L. Systolic blood pressure: 158 (141, 167) mmHg, diastolic blood pressure: 94 (83, 98) mmHg.

Hb, hemoglobin; HCT, hematocrit; T2DM, type 2 diabetes; TT, total testosterone; TTh, testosterone therapy.

median (IQR) HCT = 44 (43–46) % and median (IQR) Hb = 14.5 (14.1–14.9) g/dL at baseline; median (IQR) HCT = 49 (48–50) % and median (IQR) Hb = 14.9 (14.7–15.3) g/dL at final assessment. Table 1 demonstrates HCT and Hb levels at baseline and the fixed time points during follow-up in the total cohort on TTh, as well as subgroups based on baseline characteristics (smoking status and T2DM). Both HCT and Hb increased significantly at each time point ($p < 0.0001$, sign-rank test) compared with baseline in the total cohort as well as subgroups.

Separate multiple regression analyses showed that change in HCT at final assessment was associated with baseline HCT (coefficient [c] = −0.95, 95% confidence intervals [CI] = −1.05 to −0.87, $p < 0.001$) and baseline TT (c = −0.11, 95% CI = −0.21 to −0.016, $p = 0.023$), while change in Hb at final assessment was associated with baseline Hb (c = −0.53, 95% CI = −0.60 to −0.46, $p < 0.001$), baseline HCT ($c = 0.021$, 95% CI = −0.40 to −0.0018, $p = 0.032$), baseline TT (c = −0.026, 95% CI = −0.048 to −0.0043, $p = 0.019$), and follow-up ($c = 0.0035$, 95% CI = 0.0025–0.0044, $p < 0.001$). Age, smoking status, and T2DM were not associated with change in either HCT or Hb.

Table 2 shows the median calculated HCT/Hb ratio in the total cohort and subgroups (stratified by median baseline age, Hb, HCT, serum TT, as well as smoking and diabetes status). The baseline HCT/Hb ratio was 3.03 in the total cohort. The HCT/Hb ratio increased significantly ($p < 0.0001$, sign-rank test) at every time point (sign-rank test, $p < 0.0001$) compared with baseline in the total cohort of men on TTh and all the subgroups (Table 2). Table 2 also presents the number (and %) of men with increasing and decreasing HCT/Hb ratio. After 48, 72, and 96 months of TTh, >90% of men had an increasing HCT/Hb ratio.

We now wish to confirm the increased HCT/Hb ratio during TTh by studying the association between the change in HCT and change in Hb. A scatter plot demonstrating the association between baseline HCT and Hb ($c = 3.03$, 95% CI = 2.75–3.31) is shown (Fig. 1). We then determined the association between change in HCT and change in Hb after 48 (Fig. 2), 72, and 96 months of TTh (12-month follow-up data were omitted as the HCT continued to rise after that time point). Figure 2 (footnote table) shows the results of the linear regression; the c and intercept values were higher than those obtained from the linear regression between baseline HCT and Hb, with no overlap between the 95% CI seen in Figure 1 (footnote table). These data and plots reinforce the findings presented in Table 2: an increase in the HCT/Hb ratio occurs in men with adult-onset TD on TTh.

Although we focused on the HCT/Hb ratio following TTh, we also had data on the 384 men who opted against TTh. The median HCT remained 48% at all time points studied [baseline (384 men), 12 months (383 men), 48 months (367 men), 72 months (342 men), 96 months (283 men), and final assessment]. Interestingly there was a slight reduction in Hb during follow-up: 14.7 g/dL (baseline), 14.7 g/dL (12 months), 14.6 g/dL (48 months), 14.6 g/dL (72 months), 14.6 g/dL (96 months), and 14.5 g/dL (final assessment). Thus, unlike in the cohort on TTh where both HCT and Hb increased at all time points, in the men opting against TTh, any change in the median HCT/Hb ratios appeared driven by changes in Hb: 3.13 (baseline), 3.13 (12 months), 3.13 (48 months), 3.11 (72 months), 3.15 (96 months), and 3.16 (final assessment). As the baseline characteristics of the cohorts (men on TTh and men opting against TTh) varied, we avoided intercohort comparisons.

Discussion

In this study, we characterized the changes in HCT and Hb associated with TTh (TU) in men with adult-onset TD. At the final assessment, median HCT and Hb increased 5% and 0.4 g/dL, respectively. We also presented the changes in HCT and Hb at fixed time points (12, 48, 72, and 96 months) with the changes appearing to plateau after 48 months of treatment. The HCT/Hb ratio of 3.03 at baseline (Table 2 and Fig. 1) was similar to the numeral 3.0 that is often quoted in the literature.[37–39] However, while on TTh, the HCT/Hb ratio significantly increased with the ratios increasing in >90% of men on TTh for 48, 72, and 96 months. The change in HCT/change in Hb ratio was also significantly greater while on TTh (Fig. 2).

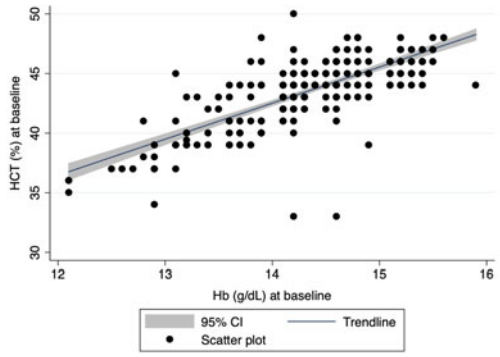
Studies determining the long-term effects of TTh on both HCT and Hb are scarce. Wang et al. studied HCT and Hb concentrations in 123 men after 36 months of treatment with long-term testosterone gel.[41] HCT and Hb levels appeared to show a dose-related increase over 12 months before plateauing. Aversa et al. showed in an randomized-controlled trial (RCT) that TU (40 men with the metabolic syndrome or adult-onset TD) led to HCT and Hb increases after 12 and 24 months. HCT and Hb increased from baseline figures of 44.0 ± 3.0% and 14.9 ± 1.2 g/dL by 3.5 ± 3.0% and

Table 2. Hematocrit/hemoglobin ratios at baseline and after 12, 48, 72, and 96 months of testosterone therapy together with the proportion of men with increasing/decreasing ratios at each time point

	Baseline HCT/Hb ratio	12 months TU			48 months TU			72 months TU			96 months TU		
		HCT/Hb ratio	Increase (%)	Decrease (%)	HCT/Hb ratio	Increase (%)	Decrease (%)	HCT/Hb ratio	Increase (%)	Decrease (%)	HCT/Hb ratio	Increase (%)	Decrease (%)
	Median (IQR)	Median (IQR)			Median (IQR)			Median (IQR)			Median (IQR)		
Total cohort	3.03 (2.97–3.10)	3.15 (3.07–3.22)	276/353 (78.2%)	33/353 (9.3%)	3.22 (3.14–3.29)	301/313 (96.2%)	9/313 (2.9%)	3.20 (3.14–3.27)	264/279 (94.6%)	15/279 (5.4%)	3.20 (3.12–3.24)	198/207 (95.7%)	9/207 (4.3%)
Age <60 years	3.02 (2.96–3.11)	3.15 (3.06–3.22)	128/170 (75.3%)	16/170 (9.4%)	3.22 (3.15–3.29)	151/157 (96.2%)	6/157 (3.8%)	3.20 (3.14–3.27)	138/145 (95.2%)	7/145 (4.8%)	3.20 (3.11–3.25)	106/108 (98.1%)	2/108 (1.9%)
Age ≥60 years	3.03 (2.97–3.10)	3.15 (3.07–3.22)	148/183 (80.9%)	17/183 (9.3%)	3.22 (3.14–3.29)	150/156 (96.2%)	3/156 (1.9%)	3.21 (3.15–3.27)	126/134 (94.0%)	8/134 (6.0%)	3.20 (3.12–3.24)	92/99 (92.9%)	7/99 (7.1%)
Hb <14.5 g/dL	3.05 (2.95–3.12)	3.17 (3.09–3.22)	143/172 (83.1%)	13/172 (7.6%)	3.24 (3.20–3.31)	150/153 (98.0%)	3/153 (2.0%)	3.24 (3.20–3.31)	127/131 (96.9%)	4/131 (3.1%)	3.22 (3.19–3.27)	102/105 (97.1%)	3/105 (2.9%)
Hb ≥14.5 g/dL	3.03 (2.98–3.09)	3.12 (3.05–3.20)	133/181 (73.5%)	20/181 (11.0%)	3.18 (3.10–3.24)	151/160 (94.4%)	6/160 (3.8%)	3.18 (3.08–3.24)	137/148 (92.6%)	11/148 (7.4%)	3.14 (3.06–3.20)	96/102 (94.1%)	6/102 (5.9%)
Hct <44%	2.95 (2.90–3.01)	3.14 (3.05–3.22)	104/113 (92.0%)	6/113 (5.3%)	3.24 (3.19–3.31)	109/110 (99.1%)	1/110 (0.9%)	3.25 (3.19–3.31)	98/98 (100.0%)	0/98 (0.0%)	3.22 (3.18–3.27)	89/89 (100.0%)	0/89 (0.0%)
Hct ≥44%	3.08 (3.01–3.14)	3.15 (3.08–3.22)	172/240 (71.7%)	27/240 (11.3%)	3.20 (3.13–3.27)	192/203 (94.6%)	8/203 (3.9%)	3.20 (3.11–3.24)	166/181 (91.7%)	15/181 (8.3%)	3.16 (3.08–3.24)	109/118 (92.4%)	9/118 (7.6%)
TT <10.05 nmol/L	3.08 (2.97–3.15)	3.17 (3.09–3.25)	120/159 (75.5%)	19/159 (11.9%)	3.24 (3.18–3.29)	121/125 (96.8%)	3/125 (2.4%)	3.22 (3.17–3.29)	102/107 (95.3%)	5/107 (4.7%)	3.22 (3.14–3.27)	67/67 (100.0%)	0/67 (0.0%)
TT ≥10.05 nmol/L	3.02 (2.96–3.08)	3.13 (3.06–3.20)	156/194 (80.4%)	14/194 (7.2%)	3.21 (3.14–3.27)	180/188 (95.7%)	6/188 (3.2%)	3.20 (3.12–3.27)	162/172 (94.2%)	10/172 (5.8%)	3.18 (3.09–3.24)	131/140 (93.6%)	9/140 (6.4%)
Smokers	3.02 (2.95–3.09)	3.15 (3.07–3.25)	104/135 (77.0%)	19/135 (14.1%)	3.22 (3.17–3.29)	117/119 (98.3%)	1/119 (0.8%)	3.24 (3.16–3.29)	102/103 (99.0%)	1/103 (1.0%)	3.20 (3.14–3.24)	86/88 (97.7%)	2/88 (2.3%)
Nonsmokers	3.05 (2.98–3.12)	3.15 (3.07–3.20)	172/218 (78.9%)	14/218 (6.4%)	3.22 (3.14–3.27)	184/194 (94.8%)	8/194 (4.1%)	3.20 (3.13–3.27)	162/176 (92.0%)	14/176 (8.0%)	3.19 (3.08–3.24)	112/119 (94.1%)	7/119 (5.9%)
Men with T2DM	3.05 (2.99–3.15)	3.14 (3.06–3.20)	107/148 (72.3%)	20/148 (13.5%)	3.20 (3.13–3.27)	116/121 (95.9%)	3/121 (2.5%)	3.20 (3.12–3.26)	94/100 (94.0%)	6/100 (6.0%)	3.18 (3.08–3.24)	68/74 (91.9%)	6/74 (8.1%)
Men without T2DM	3.02 (2.96–3.09)	3.15 (3.08–3.22)	169/205 (82.4%)	13/205 (6.3%)	3.22 (3.17–3.29)	185/192 (96.4%)	6/192 (3.1%)	3.22 (3.15–3.28)	170/179 (95.0%)	9/179 (5.0%)	3.20 (3.13–3.25)	130/133 (97.7%)	3/133 (2.3%)

HCT/Hb ratios were significantly higher ($p < 0.0001$, sign-rank test) after 12, 48, 72, and 96 months of TTh than baseline values in the total cohort and subgroups (age, Hb, HCT, and TT stratified by median baseline values and smoking and T2DM status).

TU, testosterone undecanoate.

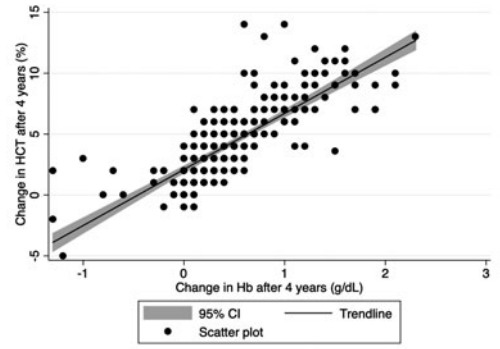


Superimposition of the data points is present in the above 2-dimensional scatterplot.

Linear regression analysis between HCT and Hb at baseline.

	c (95% CI), p	Intercept (95% CI), p	
Dependent variable: Baseline HCT (%)			n=353
Independent variable: Baseline Hb (g/dL)	3.03 (2.75, 3.31), p<0.001	0.13 (-3.90, 4.17), p=0.95	R^2 = 0.56

FIG. 1. A scatter plot and trend line demonstrating the association between HCT and Hb at baseline. HCT, hematocrit; Hb, hemoglobin.



Superimposition of the data points is present in the above 2-dimensional scatterplot.

Linear regression analyses between change in HCT (dependent variables) and change in Hb (independent variables) at 48 (scatterplot presented above), 72 and 96 months.

	c (95% CI), p	Intercept (95% CI), p	
Dependent variable: HCT (%) - 48 months			n=313
Independent variable: Hb (g/dL) - 48 months	4.60 (4.21, 5.00), p<0.001	2.10 (1.82, 2.37), p<0.001	R^2 = 0.56
Dependent variable: HCT (%) - 72 months			n=279
Independent variable: Hb (g/dL) - 72 months	5.13 (4.70, 5.56), p<0.001	1.77 (1.45, 2.08), p<0.001	R^2 = 0.56
Dependent variable: HCT (%) - 96 months			n=207
Independent variable: Hb (g/dL) - 96 months	4.56 (4.09, 5.04), p<0.001	1.99 (1.59, 2.38), p<0.001	R^2 = 0.64

FIG. 2. A scatter plot and trend line demonstrating the association between change in HCT and Hb after 48 months of TTh. TTh, testosterone therapy.

1.4 ± 1.05 g/dL (12 months) and $3.1 \pm 3.5\%$ and -0.3 ± 1.1 g/dL (24 months), respectively.[42] The results after 24 months are similar to our results. We showed a similar pattern after 12, 48, 72, and 96 months, an increase in HCT being a common effect of TU.[42]

An increase in erythropoiesis is a possible mechanism for the increased HCT and Hb. This could occur with increased erythropoietin and/or direct stimulation of bone marrow erythroblasts, as well as iron incorporation into erythrocytes.[27,28,31–33] Lundby et al. treated eight healthy subjects with baseline HCT of $42.0\% \pm 3.0\%$ and Hb of 14.2 ± 6.2 g/dL with erythropoietin, which stimulates the erythroid precursor cells located in the bone marrow.[43] After 12 weeks of treatment, the HCT and Hb concentrations increased to $49.0\% \pm 3.0\%$ and 17.1 ± 5.1 g/dL.[43] Their observed increase in Hb in comparison with HCT in this situation appears much higher than our own study observations, although subjects were given supplemental iron before and throughout follow-up, which may have influenced outcomes. The role of the androgen receptor CAG repeat polymorphism in mediating TTh-associated HCT change was studied by Stanworth et al. in the TIMES2 substudy.[44] It was noted that neither androgen CAG repeats nor change in serum TT levels was associated with change in HCT. Interestingly, in contrast to our findings, baseline HCT appeared positively correlated with change in HCT.[44] We cannot explain the varied findings observed; even after 12 months of follow-up (as in the TIMES2 study), the association between change in HCT and baseline HCT was negative ($c = -0.63$, 95% CI $= -0.72$ to -0.53, $p < 0.001$, $n = 346$). It must also be stated that changes in fluid status (5 cases of peripheral edema in the 23 men experiencing adverse cardiovascular events that led to the trial being discontinued prematurely) in men on TTh, as suggested by the Testosterone in Older Men with Mobility Limitations (TOM) trial, do not provide an explanation for the changes in HCT, Hb, and HCT/Hb ratio seen in our study following TTh.[8]

Our results suggest that the association between TTh and increased HCT and Hb, in addition to erythropoiesis, may also be mediated by other mechanisms. One mechanism could involve an increase in the life span of the erythrocyte associated with TTh in men with adult-onset TD. Erythrocytes are usually destroyed by macrocytes via erythrophagocytosis in the splenic and hepatic sinusoids after around 120 days, although variation in this duration can occur.[45] It has been suggested that oxidative stress, by damaging the cell

membrane and cytoplasm of the erythrocyte, shortens life span.[46,47] However, mechanisms such as eryptosis (considered a suicidal cell death due to hyperosmolarity, oxidative stress, and exposure to xenobiotics) may occur, although earlier than erythrophagocytosis.[48] The process of erythrophagocytosis appears mediated by a dynamic balance between phosphatidylserine (prophagocytic)[45] on the inner layer of the cell membrane and the membrane protein CD47 (antiphagocytic).[49] Angelova et al. studied the impact of TTh on erythrocytes and found compositional changes in the cell membrane.[34] This change may possibly lead to lengthening the erythrocyte life span. Around 20% of Hb is lost from erythrocytes via vesiculation, which removes damaged membrane constituents.[36,50,51] As this appears to be a gradual process,[51] increased life span would lead to greater Hb loss.

Thus, we can speculate that reduced degradation of erythrocytes, possibly associated with TTh due to changes in membrane structure, could result in an increased HCT and increased HCT/Hb ratio, as observed in our analysis. In the event of the above phenomena, it is important to consider the clinical implications as increased erythrocyte life span may result in HbA1c values that do not represent the glycemic status of the patient. The testosterone for diabetes mellitus (T4DM) RCT showed that TTh (TU) treatment and lifestyle measures in 504 obese/overweight men with impaired glucose tolerance or newly diagnosed T2DM aged 50–74 years over a 24-month period (compared with 503 men on placebo and lifestyle measures) were associated with significantly lower glucose values (2-h glucose tolerance test).[52] Interestingly, however, no difference in HbA1c between the two study arms was observed. The authors of the T4DM trial speculated whether increased erythrocyte longevity could have contributed to this finding.[52] It must be noted that unlike our longitudinal study where no man was seen to have an HCT >52%, 22% (106 men) of the men on TU (1% (6 men) in the placebo group) had at least a single HCT ≥54%.[52] It must be stated that TU was discontinued in only 23 men due to two HCT values ≥54%.[52] Change in Hb has not yet been reported by the T4DM investigators.

An increase in viscosity associated with higher HCT is likely to affect blood flow and perfusion.[16] This effect would be dependent on many factors such as erythrocyte age, deformability, and morphological changes associated with glycemic status.[16] Thus, the optimal HCT may vary for the various conditions leading to increased HCT, as they may have differing effects on the condition of the erythrocyte.

Our longitudinal registry study has strengths and weaknesses. Compliance was absolute as the TU was administered in the practice. Follow-up was relatively long, and data on HCT and Hb were almost complete. We did not have data on erythrocyte count or estrogen levels, which could be related to erythrocytosis.[53] It must be emphasized that our findings are perhaps specific for the cohort studied and TU therapy. The effects could be different with other testosterone preparations with varying half-lives.[17] Finally, our findings were observational and did not investigate mechanisms of TTh-associated erythrocytosis.

Conclusion

In this longitudinal study of 353 men with adult-onset TD treated with TU, we studied the changes in HCT and Hb at fixed follow-up time points: 12, 48, 72, and 96 months. Both HCT and Hb increased at every time point (c.f. baseline) in the total cohort and subgroups based on baseline characteristics. Baseline HCT and Hb levels were inversely associated with change in HCT and Hb, respectively, at all the time points studied. At baseline, the HCT/Hb ratio of 3.03 (95% CI: 2.97–3.10) was similar to the expected value of 3.0. Importantly, the median HCT/Hb ratio increased in the total cohort and selected subgroups following TU, with over 90% of the men demonstrating an increase in value. We suggest that the increase in the ratio may be due to the increased erythrocyte life span as speculated by the investigators of the T4DM study.[52] It is essential that the effects of the TTh-associated changes in HCT, Hb, and HCT/Hb ratio on outcomes such as erythrocyte life span, blood flow characteristics such as peak systolic velocity, CVD, and mortality be evaluated via prospective studies.[16,54]

Authors' Contributions

N.L., A.M., C.S.K., and S.R.: design of study, data analysis, and preparation of the article. R.C.S. and G.H.: preparation of the article. A.H. and K.S.H.: patient recruitment, data collection, and preparation of the article. P.D.: transposing the data and maintaining the database. F.S.: maintaining the database, design of study, and preparation of the article.

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S.R. has received research grants, travel grants, and speakers' honoraria from Besins Healthcare. F.S. works as a consultant for and is a stockholder of Bayer AG. G.H. has been an occasional speaker for Bayer AG and Besins Healthcare. A.H. and K.S.H. have received research grants, travel grants, and speakers' honoraria from Bayer AG. R.C.S. has received funding from the North Staffordshire Medical Institute. C.S.K., N.L., A.M., and P.D. have no disclosures.

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Abbreviations Used

CI = confidence intervals
CVD = cardiovascular disease
HCT = hematocrit
Hb = hemoglobin
RCT = randomized-controlled trial
sTfr = soluble transferrin receptor
T2DM = type 2 diabetes
T4DM = testosterone for diabetes mellitus
TD = testosterone deficiency
TT = total testosterone
TTh = testosterone therapy
TU = testosterone undecanoate